Exogenous application with plant growth promoting rhizobacteria (PGPR) or proline induces stress tolerance in basil plants (*Ocimum basilicum* L.) exposed to water stress

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***Abstract*—** *A pot experiment was conducted to investigate the effects of plant growth promoting rhizobacteria (PGPR) like Azotobacter chrocoocum A101, Pseudomonas fluorescens, pseudomonas mendocina Palleroni 1970 and Azospirillum lipoferum N040 or proline on growth traits, photosynthetic pigments, relative water content (RWC), electrolyte leakage percent (EL%), osmoprotectants such as proline and soluble sugars, activities of antioxidant enzymes like peroxidase (POD), polyphenol oxidase (PPO) and catalase (CAT), oil percent and water use efficiency (WUE) of basil plants subjected to water stress. Plants were treated with two regimes of irrigation water, i.e., 100% of evapotranspiration (ETc) (control) and 60% of ETc and PGPR or proline. Growth traits, photosynthetic pigments, RWC, EL %, proline and soluble sugars concentrations, activities of antioxidant enzymes oil percent and water use efficiency (WUE) were significantly altered by water stress and PGPR or proline treatments. Results indicated that PGPR or proline mitigated the water stress and significantly reduced the reduction in growth traits and leaf water content as compared to non-PGPR or proline-treated water-stressed plants. Water-stressed plants treated with PGPR or proline had significant higher photosynthetic pigments, proline and soluble sugars concentrations than water-stressed plants without PGPR or proline treatments. Higher POD, PPO and CAT activities were also observed in water-stressed plants treated by PGPR or proline than water-stressed plants without PGPR or proline treatments. Furthermore, water-stressed plants treated with PGPR or proline treatments had also significant higher oil percent and WUE as compared to water-stressed plants without PGPR or proline treatments. These results are important as the potential of PGPR or proline to alleviate the harmful effects of water stress and offers an opportunity to increase the resistance of basil plants to growth under drought conditions. The protective action of PGPR was more efficient than proline.*

***Keywords*— Antioxidant system; Anatomy; Basil; Osmoprotectants; PGPR; Proline; Water stress.**

I. INTRODUCTION

In aromatic plants, growth and essential oil production are affected by various environmental factors, such as water stress (Mahajan and Tuteja, 2005). Basil (*Ocimum basilicum* L.) is an annual plant belongs to the Lamiaceae family which has been grown for its essential oil. The essential oil of basil is used to flavor foods, dental and oral products in fragrances and in medicines. The essential oil content varies between 0.2-5.2 percent. (Penuelas and Munne-Bosch, 2005). Drought, one of the environmental stresses, is the most significant factor restricting plant growth and crop productivity in the majority of agricultural fields of the world (Tas and Tas, 2007). The drought phenomenon is a chemical - physical complex, intervene in the organization of a number of large and small bio-molecules, such as nucleic acids, proteins, carbohydrates, fatty acids, hormones, ions, and nutrients (Chaves *et al.,* 2003; Dhanda *et al.,* 2004). Khalid (2006) reported that fresh and dry weights of *Ocimum* sp. were significantly decreased due to water stress application. Meanwhile, essential oil percentage, proline and sugar content increased while nutrient content decreased. Fresh and dry weights of *Ocimum basilicum* L. decreased as plant water deficit increased (Simon *et al.,* 1992). The essential oil yield and proline contents of basil (*Ocimum* sp.) increased by subjecting plants to water stress just before harvesting (Baeck *et al.,* 2001). Plant tissues exposed to environments with water deficit have generally shown reduction in cell size, and increase in vascular tissue and cell wall thickness (Pitman *et al*., 1983; Guerfel *et al*., 2009). Drought stress increases the formation of reactive oxygen species (ROS) such as H_2O_2 (hydrogen peroxide), O^{2-} (superoxide) and OH^- (hydroxyl) radicals. Excessive ROS reduction can cause oxidative stress, which damages plants by oxidizing photosynthetic pigments, membrane lipids, proteins and nucleic acids. The accumulation of stress metabolites like poly-sugars, proline, glycinebetaine (GB), abscicic acid (ABA) as well as up-regulation in synthesis of enzymatic and non-enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), ascorbic acid (AA), α-tocopherol and glutathione are some of the significant biochemical responses in higher plants under water stress (Ramachandra Reddy *et al*., 2004; Valliyodan and Nguyen, 2006; Cattivelli *et al*., 2008). A high degree of tolerance to drought stress is offered by constitutive anatomical features which are stable in higher plants and hence act as better-observed indicators (Rhizopoulou and Psaras, 2003; Kulkarni *et al*., 2008). For a

comprehensive understanding of water stress tolerance mechanisms in higher plants, aspects of physiology and cellular biochemistry should be investigated in combination with morpho-anatomical traits in order to find out the subtle links leading to better drought resistance. Such integrated traits, expressed at a higher level of organization are suggested to be quintessential in crop improvement programs.

Proline, a multifunctional amino acid that besides acting as an excellent osmolyte is also known for stabilizing sub-cellular structures such as proteins and cell membranes, scavenging free radicals, balancing cellular homeostasis and signaling events and buffering redox potential under stress conditions (Szabados and Savoure, 2009).

Plant growth promoting rhizobacteria (PGPR) are naturally soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. PGPR are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms. Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil as a way to augment growth (Yasmin *et al*., 2007). Some PGPR also elicit physical or chemical changes related to plant defense, a process referred to as 'induced systemic resistance' (ISR) (van Loon *et al*., 1998). However, fewer reports have been published on PGPR as elicitors of tolerance to abiotic stresses, such as drought.

Several strategies have been proposed to alleviate the degree of cellular damage caused by water stress and to improve crop tolerance. Among them, exogenous application of plant growth promoting rhizobacteria (PGPR) or compatible osmolytes such as proline, glycinebetaine, trehalose, etc., had gained considerable attention in mitigating the effect of stress (Ashraf and Foolad, 2007; Zahedi and Abbasi, 2015). There for, the aim of this study was to explore effects of PGPR or proline on drought tolerance in basil and to determine the interactive impacts of water stress, PGPR or proline on growth, anatomical features, oil yield and WUE in addition to osmotic components, antioxidant system and their possible role in reducing water deficit in basil plants.

II. MATERIAL AND METHOD

2.1 Plant Materials And PGPR

Seeds of basil (*Ocimum basilicum* L.) were obtained from Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Giza, Egypt. We obtained the bacterial strains; *Azotobacter chrocoocum* A101, *Pseudomonas fluorescens*, *pseudomonas mendocina* Palleroni 1970 and *Azospirillum lipoferum* N040 from Agricultural microbiology department, Faculty of Agriculture, Cairo University for research purpose.

2.2 PGPR Culture Preparation

PGPR cultures were prepared by growing the bacterial strains; *Pseudomonas fluorescens and pseudomonas mendociana* Palleroni 1970 in Luria-Bertani (LB) broth medium (Bertani *et al*., 1951) [10.0g/l Tryptone, 5.0g/l yeast extract, 10.0g/l NaCl PH 7.0]. *Azotobacter chrocoocum* A101 were grown in complete medium (Strandberg and Wilson, 1968) [peptone 10.0g/l, yeast extract 8.0g/l and NaCl 5.0g/l]. *Azospirillum lipoferum* N040 were grown in liquid nitrogen free biotin based (NFB) medium (Piccoli *et al*., 1997) [5g/l Peptone and 3g/l Beef PH 7.0]. Equal volume of the four strains suspension mixed together and the bacterial cells were pelletized by centrifugation (5000 r.p.m) for 10 min and re-suspended in sterilized tap water containing 0.025% (v/v) Tween-20 to the desired concentration (10^8cfu ml^{-1}).

2.3 Experimental Design And Treatments

Two pot experiments were conducted at Demo Experimental Farm, Faculty of Agriculture, Fayoum University (Southeast Fayoum; 29° 17'N; 30° 53'E), during the two successive seasons of 2014 and 2015. Seeds of a uniform size were washed with distilled water after surface sterilizing with a 10 % sodium hypochlorite solution. Then seeds were imbibed in water, or in the mixture of bacterial strains suspension (at 10^8cfu ml^{-1}) for 2 h on a rotary shaker at 81 rpm, air-dried, and sown immediately. The cell densities in the bacterial suspensions were adjusted to a final density of approximately 10^5cfu $seed^{-1}$. With respect to proline treatments foliar spray of water, 1 mM proline was done at early morning with a sprayer (Vol. 5 L) to run-off six times, at 35, 45 and 55 ds after sowing in the first cutting and 10, 20 and 30 ds after the first cutting in the second one. The concentrations of proline and PGPR, the number and timing of sprays, and the soaking duration were based on results from a preliminary pot trial (data not shown). To ensure optimal penetration into leaf tissues, 0.1% (v/v) Tween-20 was added to the foliar sprays as a surfactant. On the 15th march 2014 and 15th march 2015 the seeds were sown in plastic pots (32 cm in diameter) equally filled with 8 kg clay soil having pH (1:2, w/v,

soil and water solution) 7.05, EC (1:2, w/v, soil and water solution) 3.49 dS m^{-1}, $CaCO_3$ 10.81% and organic matter 3.39%. The pots were transplanted in an open greenhouse. The average day and night temperatures were 30 ± 3°C and 15 ± 2°C, respectively. The relative humidity ranged from 65.1 to 68.8%, and day-length from 13 to 14 h. All pots were arranged in complete randomized design having two irrigation water regimes [100% (control) and 60% of evapotranspiration (ETc)]. Irrigation was applied twice a week and the pots were irrigated every 2 weeks with a nutrient solution containing 150 mg l^{-1} nitrogen (N), 100 mg l^{-1}phosphorus (P), 150 mg l^{-1} potassium (K), 2.0 mg l^{-1} iron (Fe), 1.0 mg l^{-1} manganese (Mn), 0.4 mg l^{-1} boron (B), 0.1 mg l^{-1} copper (Cu), 0.1 mg l^{-1} zinc (Zn), and 0.05 mg l^{-1} molybdenum (Mo). Samples of leaves were collected at 65 d after sowing in the first cutting and 55 d after the first one in the second cutting to assess concentration of total chlorophylls, carotenoids, proline, and soluble sugars, electrolyte leakage, relative water content and activities of antioxidant enzymes. At the end of the first cutting (75 d after sowing) and second cutting (60 d after first cutting), the plants were removed from the pots, washed in water, and the length of shoots, water use efficiency (WUE) and oil percent were measured. A number of leaves and branches were counted and a leaf area was recorded by a LI-3000 portable area meter (LI-COR, Lincoln, NE, USA). The shoots and roots were weighed to record shoots and roots fresh mass and then placed in an oven run at 80 °C for 24 h. The dried shoots and roots were weighed to record shoots and roots dry mass.

2.4 Photosynthetic Pigments Determination

1 g of leaf tissue was homogenized with 50 mL acetone (100%) and then centrifuged. Absorbance values of the samples were measured at 662, 645 and 470 nm (Pelkin Elmer/Lambda 25) (Lichtenthaler and Welburn, 1983).

2.5 Total Soluble Sugars Determination

Soluble sugars were assessed by the method recommended by the Association of Official Agricultural Chemists (1990) using phenol sulphuric acid reagent method.

2.6 Proline Determination

Proline content in basil leaves was measured following the rapid colorimetric method of Bates *et al*., (1973). Proline was extracted from 0.5 g of dry leaf samples by grinding in 10 ml of 3% sulphos-alicylic acid. The mixture was then centrifuged at 10,000 × g for 10 min. Two ml of the supernatant was added into test tubes and 2 ml of freshly prepared acid-ninhydrin solution was also added. Tubes were incubated in a water bath at 90 °C for 30 min. The reaction was terminated in ice-bath. The reaction mixture was extracted with 5 ml of toluene and the vortex process was done for 15s. The tubes were allowed to stand at least for 20 min in the dark at room temperature to allow the toluene and aqueous phases to be separated. The toluene phase was then carefully collected into test tubes and toluene fraction was read at 520 nm using a UV-160AUV Visible Recording Spectrometer, Shimadzu, Japan. The proline content in the sample was determined from a standard curve using analytical grade proline.

2.7 Enzymatic Antioxidants Activities

Polyphenol oxidase activity was carried out according to Mayer *et al*., (1965). The sample of one g was homogenized in 2 ml of 0.1 M sodium phosphate buffer (PH 6.5) at 4ºC. The homogenate was centrifuged at 20,000 rpm for 15 min. The supernatant served as enzyme source and polyphenol oxidase activity was determined as given; the reaction mixture consisted of 1500 µl of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.1M catechol was added and the activity was expressed as change in absorbance at 495 nm at 30-s intervals for 3 min. The enzyme activity was expressed as changes in absorbance min^{-1} gm fresh weight of leaves.

Peroxidase was assayed spectro photochemically according to (Amako *et al.*, 1994) the assay was carried out at 25 °C in 1.0 cm light path cuvette and the reaction mixture consisted of 1500 µL sodium phosphate buffer (PH 6.5), 1000 µL pyrogallol and 480 µL H_2O_2 solution. The enzyme extract was prepared in similar way to the one used for the extract of polyphenol oxidase. After mixing the reaction was initiated by adding the enzyme extract (20 µL) and the increase in optical density at 430 nm against blank (without extract) was continuously recorded every minute (for 1 min).

Catalase activity was determined using a colorimetric assay based on the yellow complex with molybdate and H_2O_2, which was described in detail by Goth (1991) by incubating the enzyme sample in 1.0 ml substrate (65 µmol/ml hydrogen peroxide in 60 mmol/l sodium–potassium phosphate buffer, pH 7.4) at 37 °C for four minutes. The reaction was stopped with

ammonium molybdate. Absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at 374 nm against the blank.

2.8 Determination Of Electrolyte Leakage And Relative Water Content

Leaf relative water content (RWC) was measured according to (Gonzalez and Gonzalez-Vilar, 2003). Three leaves plant^{-1} were sampled from the 5th node on main stem and immediately weighed (fresh mass, FM). In order to determine the turgid mass (TM), leaves were floated in distilled water inside a closed petri dish. During the imbibitions period, leaves were weighed periodically after water on the leaf surface was gently wiped with tissue paper. At the end of the imbibitions periods, leaves were placed in a pre-heated oven at 70°C for 48 h, in order to obtain dry mass (DM). All mass measurements were made at a precision of 0.001 g. Values of FM, TM and DM were used to calculate leaf RWC using the following equation: RWC (%) = [(FM − DM) / (TM − DM)] × 100

2.9 Electrolyte Leakage

In order to assess membrane permeability, electrolyte leakage (EL%) was determined according to the method described by Korkmaz *et al.*, (2010). Leaf discs (1cm in diameter) from randomly chosen plants per replicate were taken from the middle portion of fully developed leaf and washed with distilled water to remove surface contamination. The discs were placed in individual vials containing 10 ml of distilled water. After incubating the samples at room temperature on a shaker (150 rpm) for 24 h, the electrical conductivity (EC) of the bathing solution (EC1) was determined. The same samples were then placed in an autoclave at 121 ◦C for 20 min and a second reading (EC2) was determined after cooling the solution to room temperature. The electrolyte leakage was calculated as EC1/EC2 and expressed as percent.

2.10 Water Use Efficiency (WUE) Estimation

Water use efficiency (WUE) values as g dry shoot L^{-1}of applied water were calculated for different treatments after each cutting according to the following equation: WUE=*Dry weight of shoot (g/plant)/Total water used(l/plant*). (Jensen, 1983 & Lovelli *et al.*, 2007).

2.11 Oil Percent Determination

Quantitative determinations of basil essential oil obtained from different treatments were achieved by hydro distillation during first and second cuttings. Distillation of 100 g fresh leaves was continued for 2.5-3.0 h after water boiling till no further increase in the oil was observed according to (Sajjadi, 2006; Darzi *et al*., 2012). The oil was permitted to stand undisturbed and the amount of oil obtained from plant material was calculated as follows:

Oil (%) = observed volume of oil (ml)/weight of sample (g) × 100

2.12 Anatomical Study

For observation of stem and leaf anatomy, samples were taken at the end of the second cutting in the second season from the apex of the fifth internodes' of the tallest branch and its leaf fixed in FAA solution (containing 50 cm^3 of 95% (v/v) ethanol + 10 cm^3 of formaldehyde + 5 cm^3 of glacial acetic acid + 35 cm^3 of distilled water) for 48 h. Thereafter, the samples were washed in 50% ethanol, dehydrated and cleared in tertiary butanol series, and embedded in paraffin wax. Cross sections, 25µm thick, were cut by a rotary microtome (Leitz, Wetzlar, Germany), adhered by a Haupt's adhesive, stained with a crystal violet erythrosin combination (Sass, 1961), cleared in carbol xylene, and mounted in Canada balsam. The sections were observed and documented using an upright light microscope (AxioPlan, Zeiss, Jena, Germany). Measurements were done using a micrometer eyepiece and average of five readings was calculated.

To estimate the density and size of stomata, guard cells and glandular trichomes measurements, for this purpose, 3 plants of replicates (9 plants of each treatment) were selected. Measurement and scoring were performed for two well expanded leaves (on 5th node at the apex) of each plant. One sample of epidermal cells was obtained from lower surface (abaxial side) by nail varnish technique **(**Hamill *et al*., 1992). A small area of abaxial side of leaves was covered with a thin layer of clear nail polish and placed on glass slide and left to dry after drying the leaves samples removed and observed through a light microscope (BX60, Olympus, Hamburg, Germany), equipped with a digital camera (Camedia C4040, Olympus, Hamburg, Germany) equipped with a digital photo camera (Panasonic wv-cp300) [Barbieri *et al*., 2012]. Stomata dimensions, stomata density and oil glands were measured with the AnalySIS®3.2 software program for image analysis (Olympus - Hamburg, Germany) and their frequency (n/mm^2).

2.13 Statistical analysis

Treatments were arranged in a completely randomized design with six treatments. Analysis of variance was performed using the SPSS software package to determine the least significant difference (LSD) among treatments at $P \leq 0.05$, and the Duncan's multiple range tests were applied for comparing the means (Duncan, 1955).

III. RESULTS AND DISCUSSION

3.1 Growth Traits, Oil Yield And Water Use Efficiency (WUE)

Considerable variation in growth traits, oil yield and water use efficiency (WUE) among the treatments of water stress, PGPR and proline in both cuttings and two seasons as shown (Tables 1,2 and 3). Water-stressed plants without PGPR or proline showed a significant reduction in growth traits (i.e., shoot length, number of branches plant^{-1} number and leaf area, root and shoot fresh and dry weights plant^{-1}), oil yield and WUE than non-water-stressed plants without PGPR or proline (control) in both cuttings. In general, the plant growth under water stress is reduced. The observed reduction in growth traits, oil yield and WUE in drought stress condition in this study is due to the disturbance in metabolic process of the plant including chlorophyll destruction and the cell division. Water stress causes losses in tissue water content, which reduce turgor pressure in the cell, thereby inhibiting enlargement and division of cells causing a reduction in plant growth (Shao *et al*., 2007). Moreover, water stress decreased the growth rate, stem elongation and leaf expansion (Hale and Orcutt, 1987). Water stress affects essential oil percentage and yield of essential oil differently, because water stress increases the essential oil percentage but decreases shoot biomass, therefore yield of essential oil decreases. The application of PGPR or proline significantly or insignificantly increased all growth traits, oil yield and WUE in water-stressed plants as compared to water-stressed plants without PGPR or proline (control) in both cuttings and two seasons. The maximum increase in growth traits, oil yield and WUE was observed in PGPR-treated plants as compared to proline-treated plants and the control; plants without PGPR and proline. Non-water-stressed plants with PGPR or proline had significant increases in growth traits, oil yield and WUE than non-water-stressed plants without PGPR and proline treatment (control). The increment in previous mentioned attributes for the PGPR-treated plants was particularly significant in most cases as compared to all other treatments. PGPR plays an essential role in improving crop growth during stress conditions. We have found that our PGPR strains (*Azotobacter chrocoocum* A101, *Pseudomonas fluorescens, pseudomonas mendocina* Palleroni 1970 and *Azospirillum lipoferum* N040) have ameliorative effects on basil growth, oil yield and WUE. In support of our results, significant influence of *Pseudomonas fluorescens* has obtained by Abdul Jaleel *et al*., (2007) who reported that PGPR like *Pseudomonas fluorescens* significantly improved plant growth, root length, number of leaves and fresh and dry weights in *Catharanthus roseus* under drought stress. Similar results were observed by Naderifar and Daneshian (2012) who found that PGPR improved photosynthesis may be by increasing water and nutrients absorption leading to produce more assimilate and enhance plant growth. Also, Jay *et al*., (2013) who stated that PGPR like *Mesorhizobium sp*. and *Pseudomonas aeruginosa* increased the growth of chickpea plants. As shown in Table 4 application of proline increases endogenous proline concentration under water stress conditions. This will help to protect enzymes, organelle and cell membranes by reducing the lipid peroxidation (Okuma *et al*., 2004; Islam *et al.,* 2009). Besides this proline also supplies energy for growth and survival, thereby helping the plant to tolerate stress (Hoque *et al.*, 2007) thereby improving growth traits oil yield and WUE (Tables 1, 2 and 3).

3.2 Relative Water Content (RWC)

The relative water content (RWC) parameter is considered as one of the easiest agricultural parameters that can be used to screen for plants drought tolerance. In both cuttings, it is worth mentioning here that as compare to non-water-stressed and non- PGPR or proline -treated plants (control), the water-stressed plants without PGPR or proline treatments showed a significant reduction in leaf RWC whereas application of PGPR or proline in water-stressed plants significantly reduced this reduction in leaf RWC (Table 3). Thus, it appears that PGPR or proline has indefective role for improving leaf water content in basil and the PGPR was found to be more efficient than proline. The same trend was observed in the second season. This result is in accordance with those reported by Kordi *et al.,* (2013) who reported that RWC was decreased by increasing drought intensity in basil leaves. Increases in the leaf RWC and accumulation of proline in basil plants applied with PGPR or proline under drought stress might be an adaptive feature in improving its succulence and maintaining the water balance in response to drought-induced osmotic stress.

TABLE 1

EFFECTS OF SEED SOAKING IN PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) OR FOLIAR SPRAY WITH PROLINE ON SOME GROWTH TRAITS OF BASIL (*OCIMUM BASILICUM L.*) PLANTS GROWN UNDER WATER STRESS IN 2014 AND 2015 SEASONS. MEANS± SD, N= 6. MEAN PAIRS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P≤.05) ACCORDING TO THE DUNCAN'S MULTIPLE RANGE TEST.

		Plant height (cm)		Number of branches plant^{-1}		Number of leaves plant^{-1}		Leaf area (cm^2)	
		1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting
Water regime	**protectants**	**First season 2014**							
100% ETc	**Control**	35.3±0.58c	38.7±0.58c	6.3±0.58b	10.7±0.58b	131±3.06c	185±3.06d	6.70±0.10b	6.59±0.08b
	Proline	39.0±1.00b	43.7±0.58b	8.3±0.58a	12.0±1.00a	169±3.06b	228±4.00b	7.37±0.06a	7.66±0.05a
	PGPR	41.0±1.00a	45.3±0.58a	9.0±1.00a	12.3±0.58a	195±4.16a	243±4.16a	7.47±0.06a	7.75±0.61a
60% ETc	**Control**	28.0±1.00e	33.7±0.58e	5.0±0.00c	8.0±1.00c	91±2.31e	125±5.03f	5.83±0.15c	5.80±0.06c
	Proline	33.7±0.58d	37.3±1.15d	6.3±0.58b	9.7±0.58b	121±2.31d	174±2.00e	6.63±0.06b	6.36±0.14b
	PGPR	35.0±1.00cd	38.3±0.58cd	6.7±0.58b	10.3±0.58b	132±2.00c	209±3.06c	6.73±0.06b	6.41±0.08b
		Second season 2015							
100% ETc	**Control**	37.0±1.20b	39.0±0.33b	6.7±0.34b	10.4±0.10b	149±3.85d	208±2.51b	6.60±0.10c	6.90±0.17b
	Proline	42.3±0.67a	43.9±0.19a	8.3±0.25a	12.3±0.34a	189±1.15b	267±2.65a	7.47±0.05a	7.63±0.06a
	PGPR	43.1±0.64a	44.8±0.69a	8.8±0.44a	12.5±2.12a	204±6.56a	272±2.65a	7.60±0.10a	7.73±0.06a
60% ETc	**Control**	30.7±0.58c	32.6±0.51d	5.1±0.51c	8.5±0.39c	98±4.73f	141±4.36d	5.90±0.17d	5.87±0.12d
	Proline	35.7±0.86b	37.7±0.67c	6.4±0.03b	10.0±0.33bc	142±2.65e	193±5.03c	6.63±0.05bc	6.43±0.06c
	PGPR	36.6±0.51b	38.7±1.15bc	7.1±0.63b	10.3±0.25b	161±4.50c	203±4.36b	6.80±0.10b	6.60±0.10c

TABLE 2

EFFECTS OF SEED SOAKING IN PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) OR FOLIAR SPRAY WITH PROLINE ON FRESH AND DRY WEIGHTS OF ROOT AND SHOOT OF BASIL (*OCIMUM BASILICUM L.*) PLANTS GROWN UNDER WATER STRESS IN 2014 AND 2015 SEASONS. MEANS± SD, N= 6. MEAN PAIRS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P≤.05) ACCORDING TO THE DUNCAN'S MULTIPLE RANGE TEST.

		Fresh weight of root (g plant^{-1})		Dry weight of root (g plant^{-1})		Fresh weight of shoots (g plant^{-1})		Dry weight of shoots (g plant^{-1})	
		1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting
Water regime	**protectants**	**First season 2014**							
100% ETc	**Control**	5.63±0.16b	7.05±0.09c	2.28±0.01b	3.02±0.08c	25.0±0.27c	29.2±0.58c	6.21±0.09c	6.67±0.03c
	Proline	6.24±0.63a	7.69±0.09b	2.42±0.07b	3.19±0.03b	32.3±0.79b	35.2±0.88b	7.68±0.05b	7.89±0.06b
	PGPR	6.31±0.09a	8.20±0.09a	2.70±0.10a	3.33±0.03a	34.2±0.71a	37.7±1.23a	8.19±0.05a	8.40±0.08a
60% ETc	**Control**	4.52±0.10d	5.65±0.04f	1.86±0.10d	2.66±0.04d	16.2±1.03e	20.8±0.83e	4.47±0.06e	5.27±0.03f
	Proline	4.88±0.15cd	6.67±0.12e	1.94±0.09d	3.01±0.04c	22.8±0.123d	25.5±0.91d	5.87±0.04d	6.19±0.09e
	PGPR	5.08±0.20c	6.87±0.05d	2.12±0.09c	3.13±0.02b	24.6±0.67c	26.7±1.02d	6.27±0.03c	6.51±0.02d
		Second season 2015							
100% ETc	**Control**	5.97±0.18c	7.51±0.21b	2.31±0.03c	3.06±0.15c	26.8±0.18c	30.9±0.87c	6.73±0.06c	7.98±0.09c
	Proline	6.32±0.02b	8.98±0.46a	2.54±0.02b	3.41±0.08ab	35.3±0.26b	37.3±0.50b	8.39±0.04b	8.99±0.08b
	PGPR	6.72±0.09a	8.89±0.67a	2.67±0.11a	3.55±0.12a	36.3±0.31a	38.7±0.43a	8.77±0.03a	9.31±0.10a
60% ETc	**Control**	4.70±0.06f	5.79±0.41d	1.95±0.09e	2.69±0.04d	18.7±0.04f	22.4±0.36e	5.46±0.02e	6.58±0.03e
	Proline	5.29±0.03e	6.62±0.05c	2.09±0.03d	3.09±0.19bc	23.4±0.49e	27.8±0.54d	6.48±0.03d	7.67±0.01d
	PGPR	5.49±0.05d	6.91±0.15bc	2.35±0.03c	3.20±0.33bc	24.6±0.32d	28.7±1.00d	6.85±0.18c	8.07±0.13c

3.3 Electrolyte Leakage Percent (EL %)

In two cuttings and two seasons, water-stressed plants with PGPR or proline had significant reductions in the increase in EL % induced by water-stressed plants without PGPR or proline (Table 3). The highest reduction was achieved by the PGPR in non water-stressed plants as compared to water-stressed plants (control). These results prove the efficiency of PGPR or proline in alleviation of the cell membrane injury. The maintenance of cellular membrane integrity under water stress is considered to be an integral part of the drought tolerance mechanism.

TABLE 3
EFFECTS OF SEED SOAKING IN PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) OR FOLIAR SPRAY WITH PROLINE ON OIL PERCENT, WATER USE EFFICIENCY (WUE), RELATIVE WATER CONTENT (RWC%) AND ELECTROLYTE LEAKAGE (EL%) OF BASIL (*OCIMUM BASILICUM L.*) PLANTS GROWN UNDER WATER STRESS IN 2014 AND 2015 SEASONS. MEANS± SD, N= 6. MEAN PAIRS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P≤0.05) ACCORDING TO THE DUNCAN'S MULTIPLE RANGE TEST.

		Oil content (ml 100g^{-1} fresh)		WUE (g shoot DW L^{-1} water)		RWC (%)		El (%)	
		1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting
Water	**protectants**	**First season 2014**							
100% ETc	**Control**	0.103±0.006d	0.123±0.006d	1.02±0.014	1.09±0.005f	74.1±0.98c	70.9±0.21c	50.7±0.31c	50.6±0.12b
	Proline	0.123±0.006c	0.140±0.010c	1.26±0.008	1.29±0.010e	78.3±0.54b	75.6±0.85b	47.2±0.63	46.3±0.74c
	PGPR	0.133±0.006b	0.147±0.006c	1.34±0.009	1.38±0.013c	79.7±0.92a	78.7±0.82a	46.6±0.86	46.1±0.82c
60% ETc	**Control**	0.123±0.006c	0.163±0.006b	1.01±0.013	1.35±0.008d	68.3±0.57e	65.3±0.64e	60.7±0.84a	57.1±0.81a
	Proline	0.140±0.010a	0.187±0.006a	1.33±0.009	1.58±0.023b	72.5±0.89d	69.9±0.79d	52.9±0.57	50.8±0.85b
	PGPR	0.147±0.006a	0.187±0.006a	1.42±0.006	1.66±0.005a	74.3±0.52c	71.5±0.54c	52.4±1.53	49.6±0.64b
		Second season 2015							
100% ETc	**Control**	0.120±0.010d	0.127±0.006d	0.91±0.008f	1.15±0.012f	76.3±0.75c	74.8±0.37b	54.4±1.12	52.7±1.08c
	Proline	0.140±0.010b	0.150±0.010c	1.14±0.005	1.29±0.011e	78.5±1.15b	78.7±0.31a	50.7±1.40c	50.1±0.31d
	PGPR	0.143±0.006b	0.157±0.006bc	1.19±0.005c	1.34±0.014	80.3±0.77a	79.4±0.53a	47.4±0.49	48.4±1.08e
60% ETc	**Control**	0.133±0.006c	0.167±0.006ab	1.03±0.004e	1.58±0.008c	70.0±1.03e	69.4±0.44d	59.0±0.91a	58.7±0.72a
	Proline	0.153±0.006a	0.163±0.006ab	1.23±0.005	1.84±0.001	73.9±1.43d	72.4±0.86c	56.0±1.08	55.8±0.84b
	PGPR	0.163±0.006a	0.173±0.012a	1.30±0.033a	1.94±0.030a	75.1±0.76c	73.1±0.34c	55.6±1.66	54.2±1.14b

3.4 Photosynthetic Pigments

Regardless PGPR and proline treatments, total chlorophyll and carotenoids of water-stressed plants were significantly lesser than non-water-stressed plants (Table 4). Decreases in photosynthetic pigments were may be due to destruction of chlorophyll by increased activity of chlorophyll degrading enzymes and chlorophyllase under stress condition. A similar result was reported by Baker *et al.,* (2007) who stated that drought induced reduction in photosynthesis can also be attributable to decrease in chlorophyll content. Irrespective of water stress treatment, total chlorophyll and carotenoids of PGPR or proline -treated plants were significantly higher than non-water-stressed plants without PGPR or proline in both cuttings and in the two seasons. The PGPR was more effective than proline in this regard. Chlorophyll concentration and carotenoids was increased significantly in all the bacterial strain treatments. A similar result was reported by Heidari *et al.*, (2011) who stated that inoculation of bacterial strain like *Pseudomonas* sp., *Bacillus lentus*, *Azospirillum brasilens*, increased chlorophyll content in basil (*Ociumum basilicm* L.) under water stress. In the present study, a close association between proline-induced increase in photosynthetic pigments and growth of basil plants under water stress conditions has been observed. A similar relationship between growth or net CO_2 assimilation rate and photosynthetic pigments has already been observed in canola under water stress conditions (Kauser *et al*., 2006).

3.5 Soluble sugars and proline concentrations

In two cuttings and two seasons, irrespective of PGPR and proline treatments, soluble sugars and proline concentrations in water-stressed plants were significantly higher than non-water-stressed plants (Table 4). A similar result was reported by Heidari *et al.,* (2011) who stated that proline content was significantly increased in basil leaves under water stress. As for the

PGPR and proline treatments, the concentrations of soluble sugars and proline in PGPR or proline -treated plants were significantly higher than non- stressed plants without PGPR or proline (control) in most cases. PGPR was found to be more efficient than proline in this respect. Maximum soluble sugars concentration was noticed in water-stressed plants with PGPR as compared to proline-treated water-stressed plants and the control in spite of the significant increase in soluble sugars concentration in proline-treated plants as compared to the control. Interaction effect of water stress and bacterial strain were found to be significant for proline content and soluble sugars (Table 4).This can be related to the evidence that the bacterial strain supplied to basil plants interacts with the water stress tolerance of the plants. Proline, sucrose**,** and other organic sugars in quinoa contribute to osmotic adjustment during stress and protect the structure of macromolecules and membranes during extreme dehydration (Prado *et al.*, 2000). Cellular proline accumulates from about 5% of the amino acid pool under normal conditions up to 20–80% under stress due to increased synthesis and decreased degradation in many plant species (Kavi Kishor *et al.*, 2005) to enhance plant tolerance by reducing ROS damage. The mechanism by which proline reduces ROS damage and promoting plant resistance is that proline reduces water stress by detoxification of ROS produced as a result of water deficit. Proline may physically quench singlet oxygen or react directly with hydroxyl radicals (Siripornadulsil *et al.*, 2002). These reactions result in a more reducing cellular environment (higher carotenoids levels; Table 4). Proline is a compatible osmolyte, is not charged at neutral pH and is highly soluble in water. It can drive influx of water or reduce the efflux. This provides the turgor (higher RWC; Table 3) that is necessary for cell expansion.

TABLE 4

EFFECTS OF SEED SOAKING IN PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) OR FOLIAR SPRAY WITH PROLINE ON THE LEAF CONCENTRATIONS OF PHOTOSYNTHETIC PIGMENTS, TOTAL SOLUBLE SUGARS (TSS) AND PROLINE CONCENTRATIONS OF BASIL (*OCIMUM BASILICUM L.*) PLANTS GROWN UNDER WATER STRESS IN 2014 AND 2015 SEASONS. MEANS± SD, N= 6. MEAN PAIRS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P≤0.05) ACCORDING TO THE DUNCAN'S MULTIPLE RANGE TEST.

		Total chlorophyll concentration (mg g^{-1} FW)		Carotenoids concentration (mg g^{-1} FW)		TSS concentration (mg g^{-1} DW)		Proline concentration (mg g^{-1} DW)	
		1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting
Water	**protectants**	**First season 2014**							
100%ETc	**Control**	1.03±0.045f	1.30±0.037d	0.78±0.020d	0.87±0.023d	10.3±1.40e	9.3±0.808d	0.55±0.009f	0.61±0.015e
	Proline	1.30±0.066d	1.56±0.097b	0.87±0.045c	1.11±0.043c	11.7±1.40e	12.6±1.400c	0.68±0.009d	0.84±0.027d
	PGPR	1.40±0.043c	1.60±0.070ab	0.91±0.065c	1.17±0.047c	14.9±1.40d	13.5±0.808c	0.61±0.018e	0.82±0.016d
60%ETc	**Control**	1.15±0.015e	1.43±0.040c	1.11±0.065b	1.14±0.055c	16.8±0.00c	14.0±1.400c	0.95±0.016a	1.23±0.016a
	Proline	1.49±0.030b	1.69±0.040a	1.27±0.026a	1.25±0.048b	20.1±1.40b	18.2±0.000b	0.83±0.018b	1.10±0.021b
	PGPR	1.64±0.037a	1.70±0.036a	1.28±0.050a	1.42±0.028a	22.4±1.04a	20.1±0.808a	0.77±0.016c	1.02±0.024c
		Second season 2015							
100%ETc	**Control**	1.31±0.085d	1.16±0.047d	0.80±0.029d	1.17±0.029c	11.2±1.40d	12.1±0.81c	0.58±0.018d	0.64±0.009e
	Proline	1.47±0.068c	1.31±0.082c	1.12±0.070c	1.36±0.046b	14.0±1.40c	14.9±0.81b	0.70±0.017c	0.86±0.040d
	PGPR	1.52±0.074bc	1.42±0.028b	1.20±0.037b	1.44±0.068b	14.9±0.81c	15.9±0.81b	0.64±0.024cd	0.83±0.024d
60%ETc	**Control**	1.48±0.088c	1.32±0.035c	1.08±0.045c	1.41±0.016b	18.2±1.40b	16.3±1.63b	1.03±0.004a	1.24±0.024a
	Proline	1.64±0.060ab	1.45±0.046b	1.25±0.026b	1.64±0.018a	23.8±0.00a	19.6±1.40a	0.82±0.016b	1.16±0.024b
	PGPR	1.75±0.064a	1.54±0.004a	1.40±0.025a	1.69±0.050a	25.2±1.40a	21.0±1.40a	0.80±0.080b	1.09±0.018c

3.6 Antioxidant enzymes activities

Plants can activate antioxidative defense systems to protect themselves from the harmful effects of drought-induced oxidative stress. However, in two cuttings and two seasons, peroxidase, catalase and Polyphenol oxidase activities varied significantly in response to the water stress and PGPR or proline treatments (Table 5). Water stress-treated plants had significantly higher peroxidase, catalase and polyphenol oxidase activities than non-water-stressed plants. Similarly, PGPR or proline -treated plants had significantly higher peroxidase, catalase and polyphenol oxidase activities than non- PGPR or proline -treated plants. The interaction effect of water stress and PGPR or proline on peroxidase, catalase and polyphenol oxidase activities showed maximum activity in water-stressed plants treated specially PGPR treatment as compared to the non water-stressed

plants without PGPR or proline treatments. Results clearly suggest the positive role of PGPR or proline in up regulating the POD, CAT and PPO activities in basil plants under water stress. Similar effects of PGPR in increasing the antioxidant enzymes activities (GPX and APX) have also been observed in basil leaves by Heidari and Golpayegani, (2012). Results showed that PGPR or proline minimizes the negative effects of water stress (60% ETc) with evidence of enhancing leaf water potential by up regulating the endogenous production of proline, carotenoids, soluble sugars and antioxidant enzymes like SOD, CAT and PPO leading to maximization of oil yield accompanied with higher WUE.

TABLE 5

EFFECTS OF SEED SOAKING IN PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) OR FOLIAR SPRAY WITH PROLINE ON ACTIVITIES OF POLYPHENOL OXIDASE, PEROXIDASE AND CATALASE OF BASIL (*OCIMUM BASILICUM L.*) PLANTS GROWN UNDER WATER STRESS IN 2014 AND 2015 SEASONS. MEANS± SD, N= 6. MEAN PAIRS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P≤0.05) ACCORDING TO THE DUNCAN'S MULTIPLE RANGE TEST.

		Polyphenol Oxidase (U min^{-1} g^{-1})		Peroxidase (U min^{-1} g^{-1})		Catalase (U min^{-1} g^{-1})	
		1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting
Water	**protectants**	**First season 2014**					
100%ETc	**Control**	1.37±0.015f	1.71±0.009f	4.80±0.009f	5.66±0.019f	1.42±0.006f	1.47±0.027e
	Proline	1.97±0.008c	1.96±0.025e	5.43±0.005e	6.55±0.019e	1.87±0.006d	2.14±0.097c
	PGPR	1.93±0.006d	2.31±0.014c	5.73±0.009d	6.68±0.019d	1.63±0.008e	2.06±0.009d
60%ETc	**Control**	1.84±0.039e	2.10±0.054d	5.78±0.008c	6.80±0.007c	2.20±0.026c	2.15±0.014c
	Proline	2.14±0.003b	2.57±0.003b	6.26±0.025b	7.34±0.026b	2.55±0.035b	2.49±0.009b
	PGPR	2.26±0.008a	2.71±0.009a	6.66±0.018a	7.74±0.024a	2.62±0.022a	2.80±0.005a
		Second season 2015					
100%ETc	**Control**	1.39±0.012e	1.73±0.008f	4.79±0.079f	5.89±0.032e	1.42±0.023f	1.55±0.035f
	Proline	1.85±0.010d	2.12±0.031e	5.39±0.092e	6.48±0.038d	1.58±0.004e	2.08±0.040e
	PGPR	1.92±0.010c	2.34±0.005d	5.72±0.023d	6.74±0.081c	1.72±0.008d	2.23±0.049c
60%ETc	**Control**	1.84±0.050d	2.42±0.008c	6.13±0.042c	6.75±0.010c	2.13±0.012c	2.15±0.014d
	Proline	2.24±0.018b	2.83±0.008b	6.54±0.120b	7.36±0.039b	2.48±0.028b	2.49±0.009b
	PGPR	2.36±0.008a	2.97±0.034a	6.90±0.057a	7.48±0.030a	2.70±0.026a	2.70±0.016a

3.7 Anatomical studies

3.7.1 Stem

Considerable variation in anatomy of basil stem was observed between different water stress treatments with or without PGPR or proline (Table 6 and Fig. 1). Water-stressed plants without PGPR or proline (control) showed a significant reduction in dimension of stem .This was mainly due to the reduction in thickness of cortex, vascular cylinder, pith diameter and xylem vessel as compared to other treatments. Tissues exposed to environments with low water availability have generally shown reduction in cell size, and increase in vascular tissue and cell wall thickness (Guerfel *et al.,* 2009). On contrast, PGPR or proline application greatly improved all anatomical characters of stem in both water and non-water-stressed plants in comparison to water-stressed plants without PGPR or proline (control). PGPR was found to be more efficient in mitigating the adverse effects of stress by improving the stem anatomy.

3.7.2 Leaf

Table 6 and Fig. 2 show that, water-stressed plants without PGPR or proline (control) revealed a significant reduction in blade, palisade and spongy thickness while average thickness of xylem vessel was increased than non-water-stressed plants without PGPR or proline (control). The same results were obtained by Chartzoulakis *et al*. (2002) who stated that water stress resulted in a significant decrease of the thickness of almost all histological components of the mesophyll, as well as of the entire lamina thickness of avocado cultivars. Ristic and Cass (1991) reported that the vascular tissue area was decreased by low soil moisture. Yentür (2003) indicated that sclerenchyma tissue provides an advantage against the loss of water. On the other hand, the application of PGPR or proline greatly improved anatomical characters of leaf in both water and non-stressed plants as compared to non-stressed plants without PGPR or proline. The maximum blade and spongy thickness was observed

in non-water-stressed plants treated with PGPR as compared to other treatments. Similar results were presented by El-Afry *et al*. (2012).

TABLE 6

EFFECTS OF SEED SOAKING IN PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) OR FOLIAR SPRAY WITH PROLINE ON LEAF AND STEM ANATOMICAL STRUCTURE OF BASIL (*OCIMUM BASILICUM L.*) PLANTS GROWN UNDER WATER STRESS IN 2014 AND 2015 SEASONS. MEANS± SD, N= 6. MEAN PAIRS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P≤0.05) ACCORDING TO THE DUNCAN'S MULTIPLE RANGE TEST.

		Average thickness of blade (µm)	Average thickness of palisade tissue (µm)	Average thickness of spongy tissue (µm)	Average height of vascular bundle (µm)	Average width of vascular bundle (µm)	Average thickness of xylem vessel (µm)
100%ETc	Control	393±5.77b	119±1.15a	221±3.82c	280±10.0a	413±5.77c	17.5±0.00a
	Proline	403±8.77b	103±2.89c	233±2.89b	243±5.77b	690±10.0a	15.6±1.04b
	PGPR	450±10.0a	106±5.29cd	268±7.64a	240±10.0b	583±15.3b	16.5±0.87ab
60%ETc	Control	333±5.77c	89±1.44e	183±7.64d	210±10.0cd	393±5.77c	16.4±1.01ab
	Proline	390±10.0b	114±2.89b	212±2.89c	207±5.77d	413±11.55c	16.0±1.31ab
	PGPR	397±5.77b	99±1.44d	220±5.00c	223±5.77c	370±10.0d	15.0±0.00b

		Dimension of stomata		Stomata density (no. /mm^2)	Stomata aperture area (µm^2)	Glandular trichomes density (no. /mm^2)	Average of glandular trichomes area (µm^2)
		length(µm)	Width(µm)				
100%ETc	Control	30.25±0.25a	20.8±0.38a	336±2.09a	66.3±0.71a	8.9±0.15e	9797±109.4c
	Proline	26.33±0.14b	19.25±0.25b	308±2.09b	58.0±0.58b	12.1±0.22b	8894±12.5e
	PGPR	26.83±0.52b	18.42±0.76c	270±5.55d	57.3±1.84b	11.4±0.17c	9802±35.9c
60%ETc	Control	25.50±0.50c	18.75±0.50bc	311±4.18b	56.4±0.41b	10.8±0.17d	9033±31.3d
	Proline	23.92±0.52d	17.42±0.29d	279±2.09c	56.8±0.35b	11.2±0.17c	12402±26.6a
	PGPR	23.75±0.43d	17.33±0.14d	262±3.64e	49.4±0.66c	12.8±0.15a	11242±23.4b

		Dimension of stem		Average of cortex thickness (µm)	Average thickness of vascular cylinder(µm)	Average of pith diameter (µm)	Average thickness of xylem vessel (µm)
		Length (µm)	Width (µm)				
100%ETc	Control	2242±14.43b	2083±38.18cd	240±10.00a	277±5.77bc	1071±14.4cd	20.29±0.55a
	Proline	2225±43.3bc	2283±28.86ab	203±5.77bc	317±5.77a	1167±19.3ab	19.08±0.58b
	PGPR	2275±25.00b	2325±60.14a	217±5.77b	310±10.0a	1213±43.3a	19.02±0.87b
60%ETc	Control	2125±43.3d	1850±132.28e	177±5.77d	260±10.0c	1046±44.1d	18.42±0.95bc
	Proline	2183±14.43c	2050±50.0d	200±10.00c	267±15.27bc	1121±61.8bc	17.75±0.25c
	PGPR	2363±12.5a	2188±12.5bc	215±5.00b	280±0.00b	1207±6.5a	16.38±0.46d

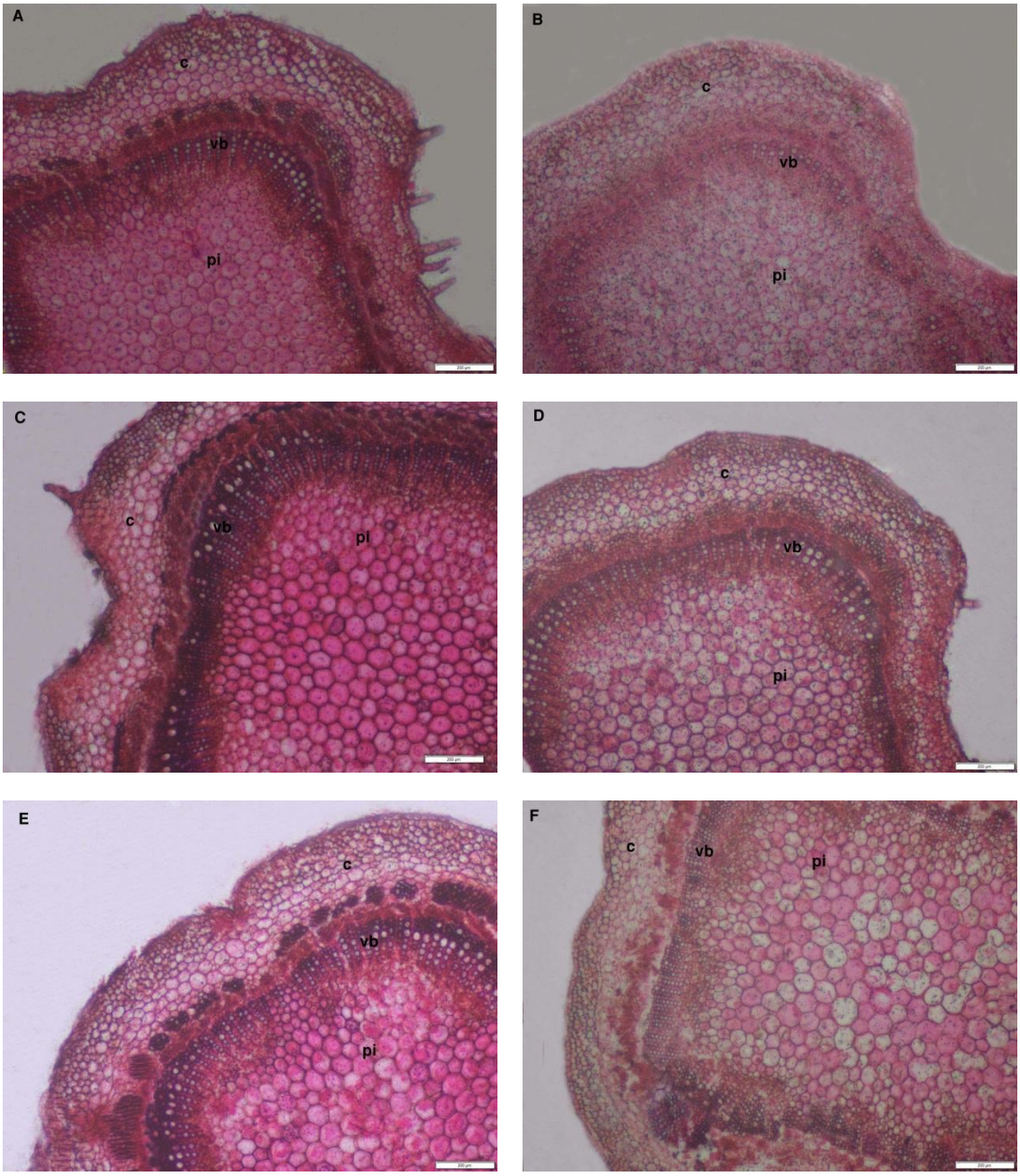

FIG. 1: TRANSECTIONS OF BASIL STEM AS AFFECTED BY APPLICATION OF PGPR OR PROLINE UNDER WATER STRESS. A) 100% of ETc; B) 60% of ETc; C) 100% of ETc+ 1mM proline; D) 60% of ETc+ 1mM proline; E) 100% of ETc+ PGPR F) 60% of ETc+ PGPR;c, cortex; pi, pith and vb, vascular bundles.

3.7.3 Stomata and glandular trichomes

Considerable variation in stomata and glandular trichomes was observed among the treatments of water stress, PGPR and proline (Table 6 and Fig. 3). Water-stressed plants without PGPR or proline showed a significant reduction in dimension of stomata, stomata aperture area, stomata density and average of oil gland area while glandular trichomes density was increased than non-water-stressed plants without PGPR or proline (control). In line with these results, Larcher (1995) who reported that leaves that developed during drought usually have smaller stomata than leaves under well watered conditions. Low stomatal density has also been reported to be a critical determinant for high WUE (Masle *et al.*, 2005), a physiological response that has been often observed in plants exposed to drought conditions (Yoo *et al*., 2010). Similarly, PGPR or proline in water-stressed plants showed the same trend in pervious attributes except average of glandular trichomes area was significantly increased as compared to non-stressed plants without PGPR or proline.

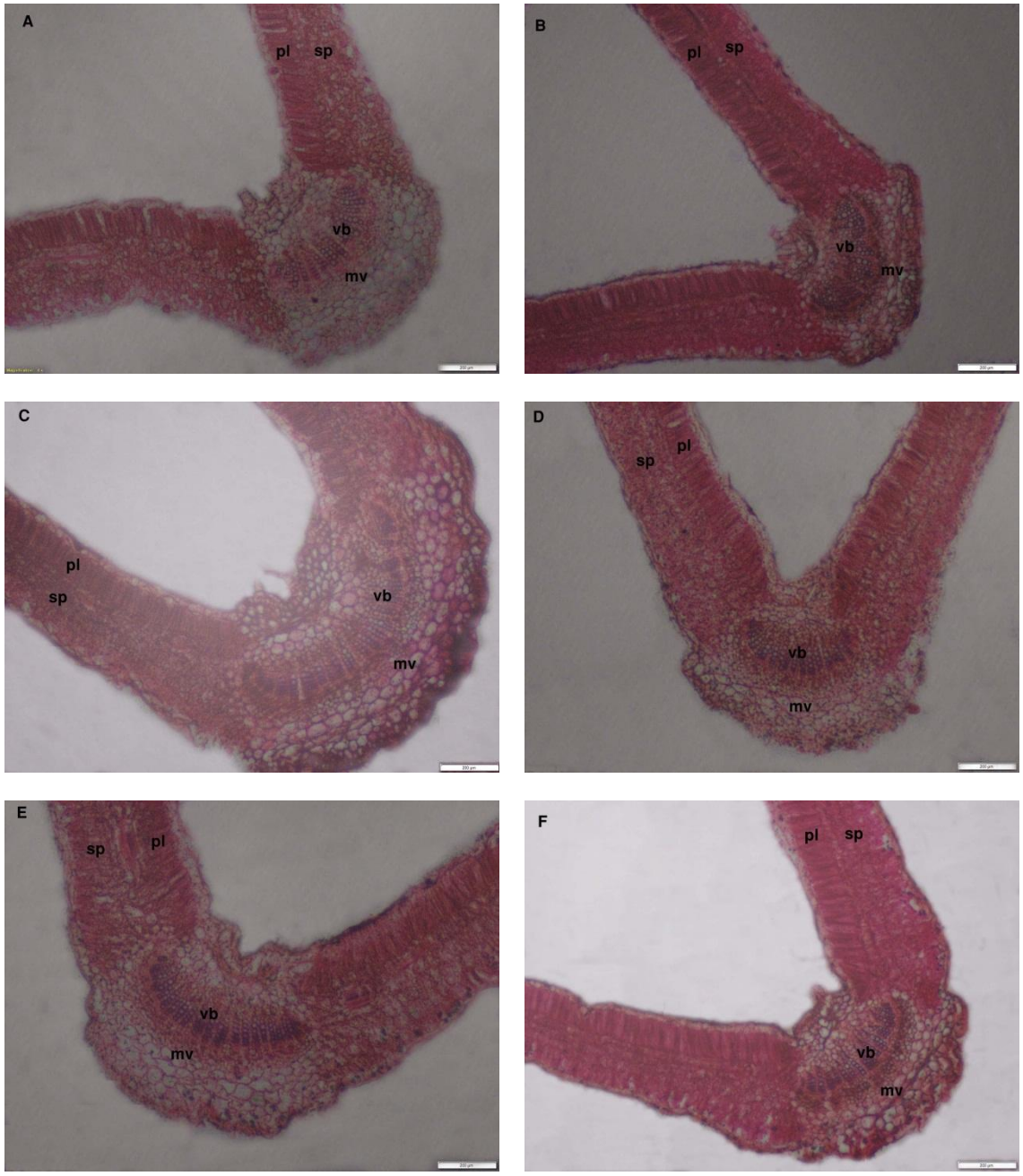

FIG. 2: TRANSECTIONS OF BASIL LEAF BLADE AS AFFECTED BY APPLICATION OF PGPR OR PROLINE UNDER WATER STRESS. A) 100% of ETc; B) 60% of ETc; C) 100% of ETc+ 1mM proline; D) 60% of ETc+ 1mM proline; E) 100% of ETc+ PGPR; F) 60% of ETc+ PGPR; sp, spongy tissue; pl, palisade tissue; mv, midvien and vb, vascular bundles.

Our resulted showed that strong correlation of leaf and stem anatomical structures with physiological traits supported the superior performance of basil plants applied with PGPR or proline under drought stress. Basil owned anatomical traits, like thicker leaves resulting from a well developed palisade and spongy tissues that would contribute to remaining green under stress and that retain higher amounts of photosynthetic pigments. The higher number of xylem vessels recorded in basil stem and leaf would improve the capabilities for water transport. We conclude that basil treated with PGPR or proline performed best under drought as it possessed a well-developed root system, improved leaf and stem anatomical characteristics, up-regulation in antioxidative defense mechanisms, osmotic balance maintenance. Our results showed that different mechanisms for drought tolerance are highly interconnected with a series of compensatory reactions that mitigate the effects of drought in a basil plants.

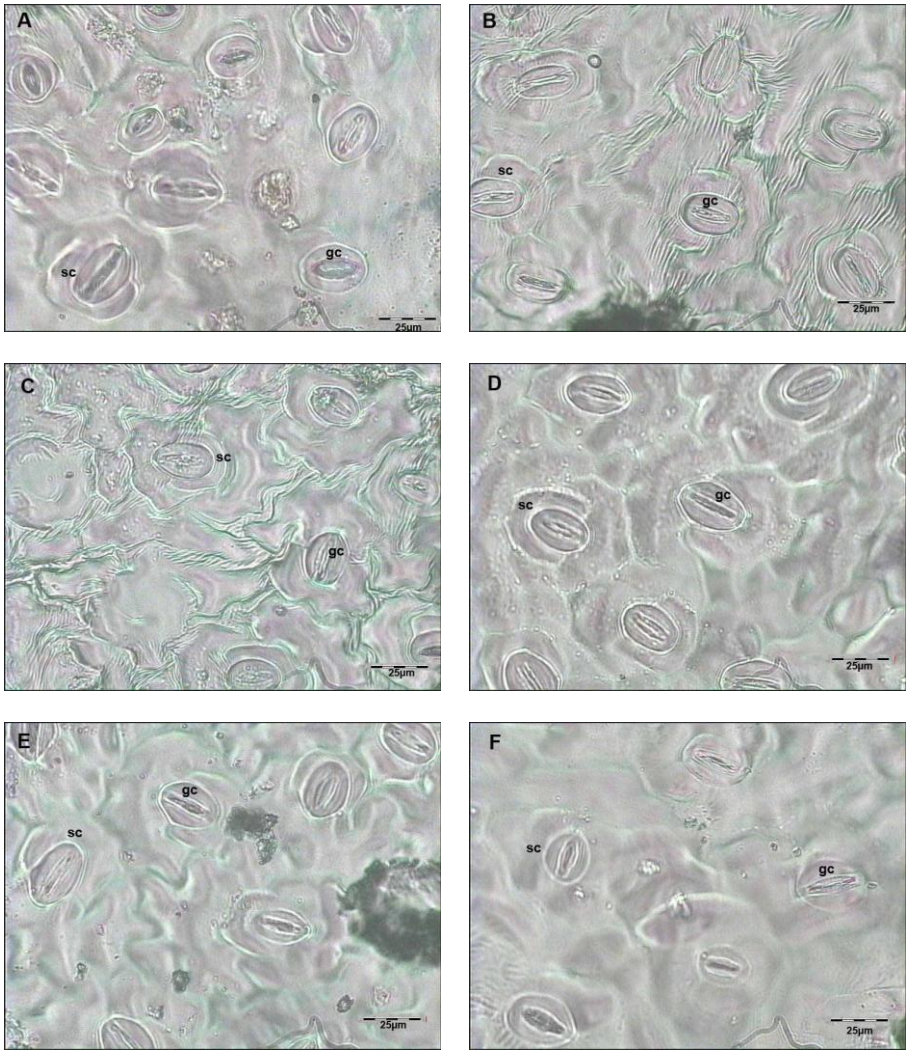

FIG. 3: ANATOMICAL FEATURES OF BASIL LEAF ABAIXAL SURFACE AS AFFECTED BY APPLICATION OF PGPR OR PROLINE UNDER WATER STRESS. A) 100% of ETc; B) 60% of ETc; C) 100% of ETc+ 1mM proline; D) 60% of ETc+ 1mM proline; E) 100% of ETc+ PGPR; F) 60% of ETc+ PGPR; gc, guard cells; sc, subsidiary cells.

IV. CONCLUSION

Application of PGPR as a seed soaking or proline as a foliar spray for basil improved the level of enzymatic and non-enzymatic antioxidants, photosynthetic capacity, osmoprotectants such as proline and soluble sugars and induced positive changes in anatomy of leaf and stem tissues under both stress and stress-free conditions, thereby increasing the tolerance of basil to drought stress and improving growth. PGPR was found to be more effective than proline.

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